USDA, ARS, National Animal Germplasm Program Goat Semen Cryopreservation Protocol
Revised January 28, 2016

Semen is collected from bucks by electroejaculation or artificial vagina and the sample is observed to make sure it is free of urine. The sperm motility, volume, and concentration are determined and the semen is diluted in one step using Tris-egg yolk-glycerol diluent (37 ºC) to 400 x 10^6 sperm/mL. Next, the samples are loaded into straws (0.25 or 0.5 mL), sealed, and cooled to 5 ºC over 2.0 hours. The samples are then frozen in liquid nitrogen vapor (4 cm above liquid nitrogen) for 7 min and plunged into the liquid nitrogen for storage.

Thawing procedure:

Frozen samples are thawed in a 37 ºC water bath for 30 sec.

Optional seminal plasma removal:

Samples can be collected and held for 24 hours at 5 ºC prior to cryopreservation using this diluent because the egg yolk concentration is low enough that coagulation should not occur (Mook and Wildeus, 2008). If this is a concern, as may be the situation with bucks which are know reactors to egg yolk, the semen sample can be washed using the same diluent (800 x g for 10 min), but without egg yolk and glycerol, to remove the seminal plasma. After removal of the seminal plasma, the sample can then be cryopreserved using the methodology described above and the diluent containing egg yolk and glycerol.

Tris-egg yolk-glycerol diluent, 100 mL volume

Tris	2.422 g
Fructose	1.0 g
Citric Acid	1.36 g
Penicillin G	0.006 g
Streptomycin sulfate	0.100 g
Egg yolk	2.5% by volume
Glycerol	2.0 % by volume

pH to 6.8-7.0

References:

Mook, J.L. Wildeus, S. 2008. Effect of egg yolk level, washing and extended pre-freeze equilibration on postthaw motility of buck semen. Southern Section American Society of Animal Science Annual Meeting. Dallas, TX.